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Does platform matter for ADA assessment? Validation and sample data revisited across multiple platforms

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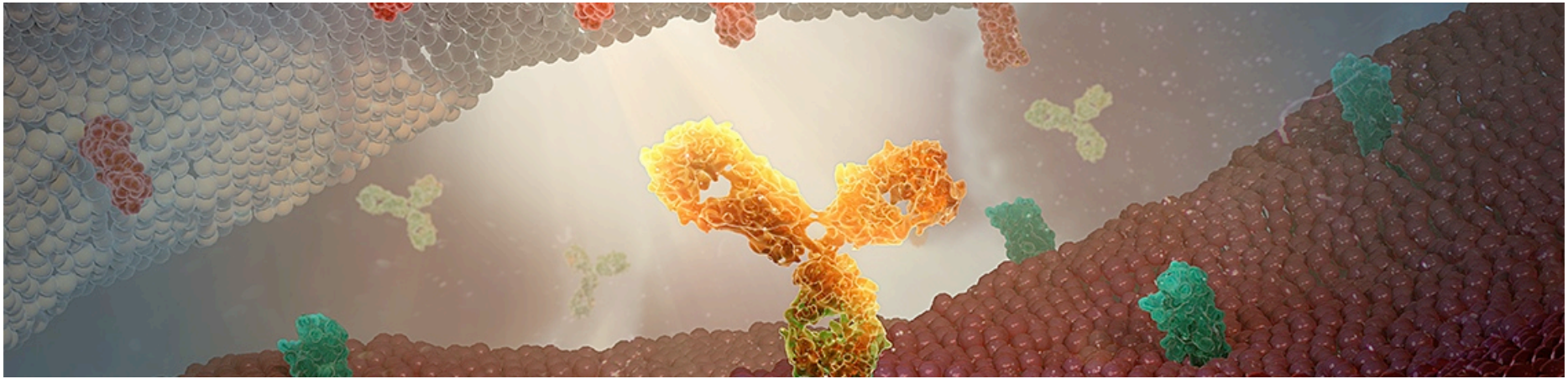


Overview

- ADA as a bioanalytical challenge
- Initiating a comparison of platforms
- Challenges and conclusions of development
- Comparison of test sample data

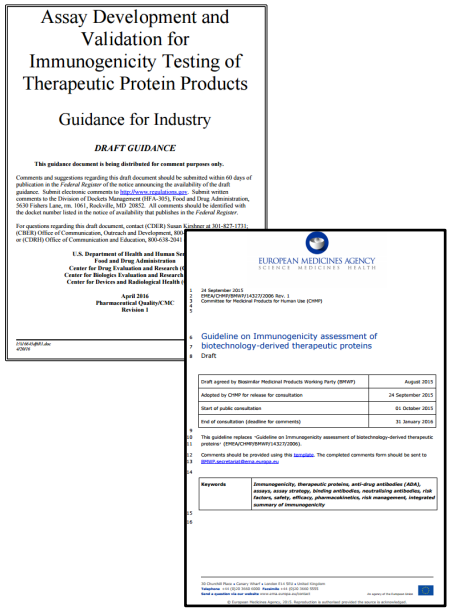
Meeting regulatory expectations for assessment of ADA is a fundamental bioanalytical challenge

- Immunogenicity is a critical component of drug development

- Agency expectations for assessment of ADA have developed significantly over the past decade and continue to challenge

- Assay validation packages are submitted at license application (potentially earlier)
  - Methods are not deemed validated until the regulators review and agree

- Strategic decisions are not made lightly – and that includes choice of platform



Specific instruments become preferred platforms for different applications

Platform	PK	PD	ADA
Gyros xP	✓	✓	
MSD S 600	✓	✓	✓
Molecular Devices Paradigm	✓	✓	

✓ = primary platform

✓ = alternate platform

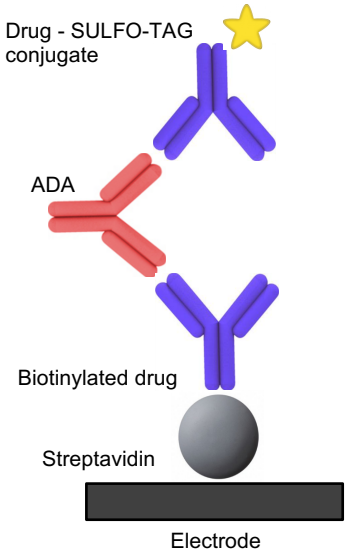
….But relying on a single vendor is potentially high-risk

- Bioveris remains a cautionary lesson

- New challenges may be better solved on alternate platforms
  - Moving beyond mAbs
    - Conjugated molecules
    - Multiple domains

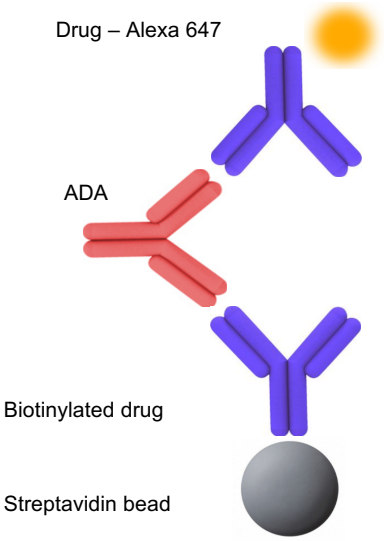
A platform comparison of MSD versus Gyrolab and AlphaLISA technologies was initiated

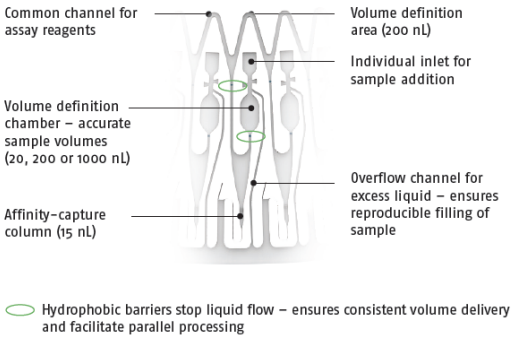
The MSD has gained significant traction in industry for analysis of immunogenicity



- Electrochemiluminescent immunoassay
  - Immunoassay complex is built on carbon electrode
  - Ruthenylated detection reagent in proximity of the electrode emits light in presence of TPA substrate in Read Buffer
- Carbon electrode has ~10x greater binding capacity than polystyrene
- Signal amplification from multiple levels of excitation per label
- Typically offers increased sensitivity and drug tolerance over ELISA

Gyrolab is a semi-automated platform using nano-fluidics on a CD micro-laboratory

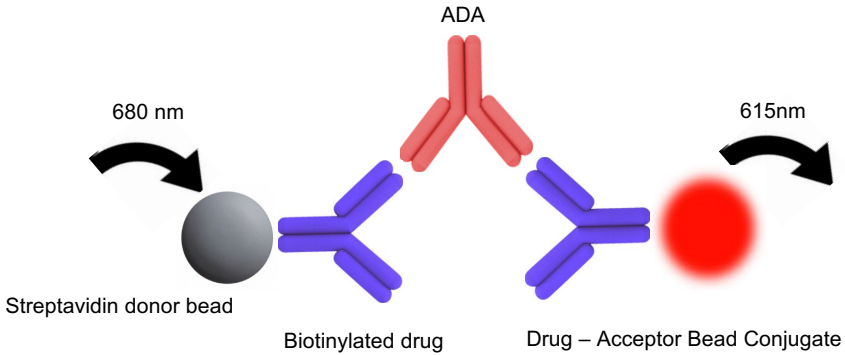




- Gyrolab ADA Solution
  - Bioaffy 200 CD
  - Bioaffy Mixing CD for acid-dissociation
  - Rexxip ADA Assay Buffer
- Updated this year to include 96 micro-laboratory mixing CD and updates to software

AlphaLISA is a bead-based no-wash solution phase immunoassay

- Amplified Luminescent Proximity Homogenous Assay
  - Solution phase assay
  - Laser excitation of donor bead leads to release of singlet oxygen molecules triggering energy transfer and spike of specific light emission from acceptor beads



- No wash protocol
  - Theoretically beneficial for detection of low affinity ADA

Method development followed a standard approach across the platforms

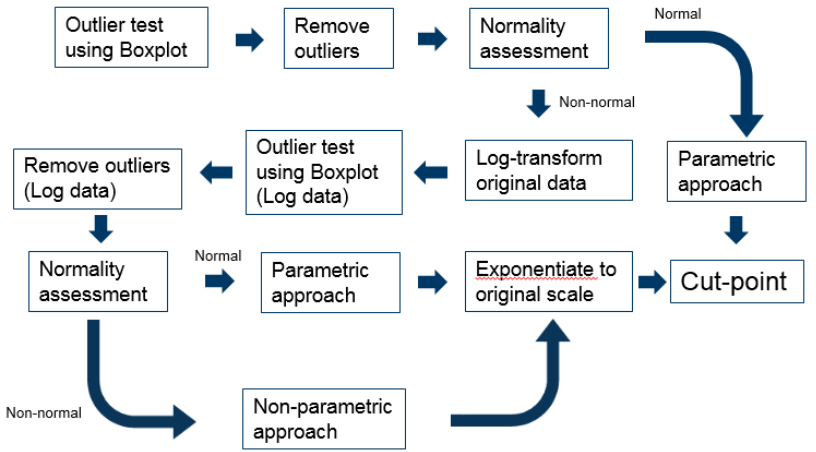
Reagent labelling

Chequerboard and assessment of MRD

Assessment of sensitivity and early look at cut-point

Assessment of drug tolerance

Cut-point assessment
N=30 individual samples



Workflows highlight operator efficiencies for Gyrolab and AlphaLISA over MSD

	MSD	Gyrolab	AlphaLISA
Day 1	Prepare samples (1:10 MRD)	Centrifuge samples/reagents	Prepare samples (1:20 MRD)
	Prepare mastermix	Prepare samples (1:4 MRD)	Prepare mastermix
	Mix samples and mastermix	Prepare mastermix	Mix samples and mastermix
	Incubate o/n at RT on shaker	Mix samples and mastermix	Incubate o/n at RT on shaker
	Block Streptavidin plate o/n	Incubate o/n at 2 to 8°C	
Day 2	Streptavidin plate wash	Load plate on xP and read	Add Donor Beads
	Sample transfer		Load plate on Paradigm and read
	Incubate 1 hour at RT on shaker		
	Wash plate		
	Add Read Buffer and read plate		

Platform-specific strengths and weaknesses are evident for each platform

Platform	Pros	Cons
MSD	• Read Time ~90 seconds • Open reagent choice enables custom optimisation • Cost	• Relatively labour intensive
Gyrolab	• Gyrolab Evaluator Software • Efficient workflow • Choice of off-the-shelf reagents • Application support	• Read Time ~50 mins • Sample handling requirements – impact on precision • Closed reagents limit custom optimisation
AlphaLISA	• Efficient workflow • Read Time ~120 seconds	• Buffer selection challenging • Additional development steps to optimise biotin Ab and bead concentrations • Expense of beads

Assay parameters indicate comparable performance between MSD and Gyrolab

	MSD	Gyrolab	AlphaLISA
Reagent Labelling	Biotin and SULFO-TAG 12:1	Biotin and Alexa 647 12:1	Biotin and Donor Beads 12:1 and 50:1
Mastermix	2.5 µg/mL Biotin 2.5 µg/mL SULFO-TAG	4 µg/mL Biotin 4 µg/mL Alexa	2 nM Biotin 10 µg/mL Acceptor 40 µg/mL Donor
MRD	1:10	1:4	1:20
Assay Buffer	1% Blocker A	Rexxip ADA	HEPES/Casein/Tween
Hook Effect	Not evident at 50,000 ng/mL	Not evident at 50,000 ng/mL	Not assessed
Precision	≤ 9.9% CV	≤ 17.5% CV	Not assessed
Sensitivity	193 ng/mL	168 ng/mL	Not assessed
Drug Tolerance	500 ng/mL PC tolerant of 10 µg/mL drug*	500 ng/mL PC tolerant of 10 µg/mL drug*	Not assessed
Cut-point Factor	1.22	2.70	Not assessed

*tolerant to between 10 µg/mL and 100 µg/mL of therapeutic

MSD data courtesy of Nick White

Sample analysis highlighted a need for further development on the Gyrolab

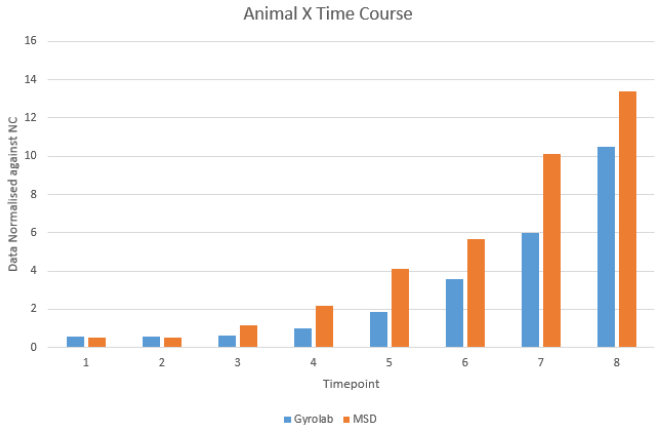
- Six Month Repeat Dose TK Study with 16 Week Treatment-Free Period
  - Four dose groups:
    - Group 1 Control
    - Group 2 50 mg/kg SC
    - Group 3 150 mg/kg SC
    - Group 4 150 mg/kg IV

- 266 samples reanalysed over 6 CD's over 2 days

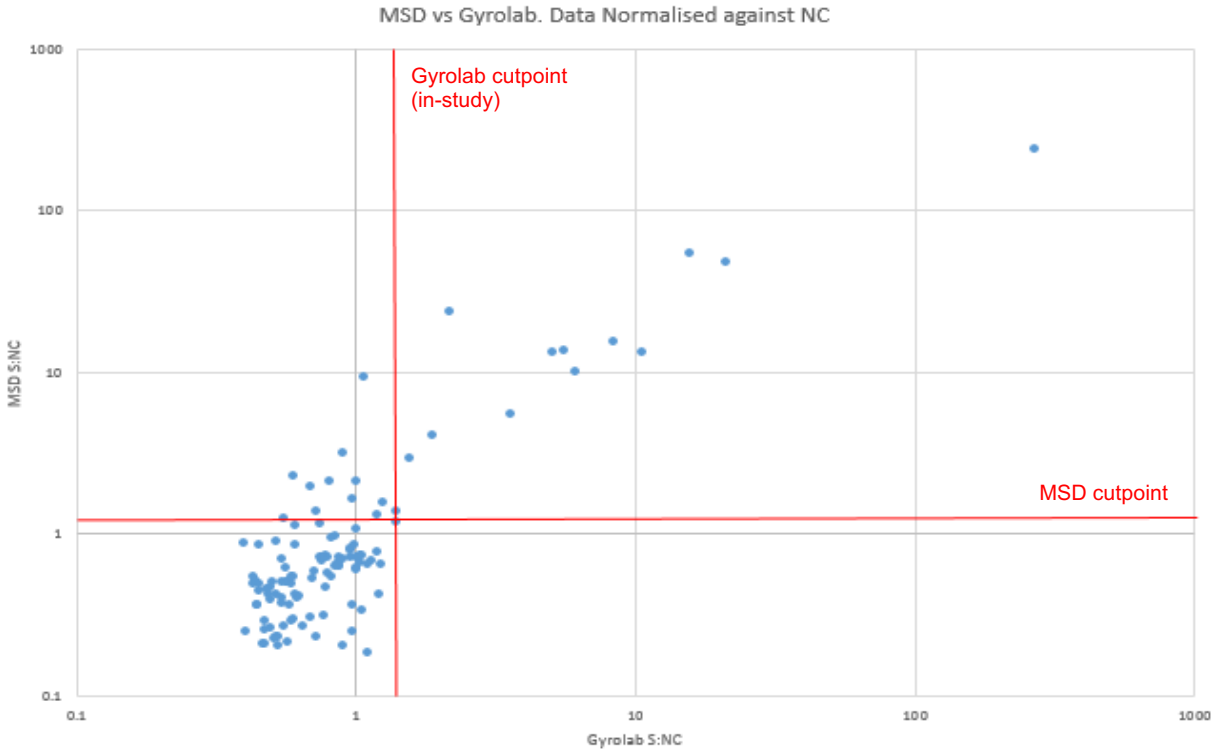
- **Assay failure → Unacceptable precision from PC samples**
  → Clearly erroneous data around low end of the assay

Rexxip ADA was substituted for Rexxip F to increase stringency

- **Development is ongoing**
  - 3 CDs of TK samples reanalysed to aid troubleshooting
  - Available data indicate increased stringency and improved assay performance
  - Positive control data show precision ≤ 9.1% CV
- Preliminary comparison against MSD data possible and some interesting correlation appears



Plotting normalised data indicates MSD detects more positive samples



- MSD detected 4 false positives
- Gyrolab detected 1 false positive
- Gyrolab PC at 50 ng/mL would be above estimated cut-point

Conclusions

- Immunogenicity assessment remains a key bioanalytical challenge
- Committing to a single platform can seem the safest option
- There remains a desire to ‘stay current’ with alternate technologies
- Comparison of platforms highlights process, cost and data as decision making variables
- There is a need to develop a significant expertise in each platform to appropriately optimise assays and thus negate data as a variable

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